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Virus in Water

I. A Preliminary Study on a Flow-Through Gauze Sampler for Recovering Virus from Waters

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A preliminary study was carried out on evaluating a flow-through gauze sampler for its efficiency in recovering virus from both fresh and seawater. An attenuated type 1 poliovirus was used as the working model. When tap water was sampled, the amounts of virus adsorbed by the gauze pads were very small, about 2% of the total number of virus particles flowing through the device. The virus adsorption and recovery increased to 15 to 19% when seawater was sampled. Addition of NaCl to tap water produced a much better effect on virus adsorption and recovery by this device, i.e., 47% of the total virus particles in each sample. The best viral elution from the pads was obtained by using buffer solution of pH 8.0 to 9.0 containing a small amount of animal serum. Repeated elutions from the pads were necessary to recover the most virus although the first eluate contained approximately 50% of the adsorbed virus. Further development of this device appears warranted, because of (i) the simplicity of the procedure, (ii) its capability of sampling large volume of water, (iii) the low cost of collecting samples, and (iv) the feasibility of obtaining a rough quantitative assessment of viral pollutants in water examined.

Examination of natural water sources of human viral pollutants has not been carried out in the United States to date because of lack of a suitable method. In the last decade, several techniques have been described and may be used for this purpose. These include two-phase polymer separation (19-21), hydroextraction (5, 21), membrane filtration (16, 24, 25), adsorbent compounds (15, 17, 22, 23), electrophoresis (2), soluble ultrafilter (8), and ultracentrifugation (1). The gauze pad method, the earliest used to examine waste water for microbial pollutants, has been almost abandoned because of lack of precision and no controlled study to document its efficacy. Hanging pads were first used by Moore (13) and met with success in locating typhoid carriers by examining sewage for the bacteria. Later it was adapted for recovery of poliovirus and other human enteric viruses in sewage (9-10). This method, in general, was used in combination with other procedures for further concentration of the virus. From limited field experience (8, 11), there was indication that this method was superior to other procedures used at that time.

Coin et al. (7) originally designed a flow-through type of gauze sampler. By this device, numerous human enteric viruses were isolated from fin-

ished and raw water in France. Coin (6) claimed that the sampler was capable of quantitatively measuring virus in water. This device was modified by Hoff, Lee, and Becker (*personal communication*). They showed that its efficiency was very low in trapping virus from fresh water samples, i.e., < 1% of the total virus in each sample when the sampled water was clear and approximately 3% when the water was mixed with mud. The sampler was evaluated in our laboratory, as part of a collaborative effort among four laboratories, in an attempt to develop standard methods for examination of water and shellfish. This report presents the preliminary findings on (i) the efficacy of the sampler in concentration and enumeration of a poliovirus in fresh and seawater and (ii) several parameters affecting the efficiency of gauze pads in sampler for abstracting viral particles from water as well as the elution process for recovering the virus from these pads.

MATERIALS AND METHODS

Virus. The LSc 2ab strain of type 1 poliovirus was used throughout this study. The stock virus was kindly supplied by the Lederle Laboratories, American Cyanamid Co., Inc. The virus was distributed in 2-ml amounts and stored at -20 C until used. It

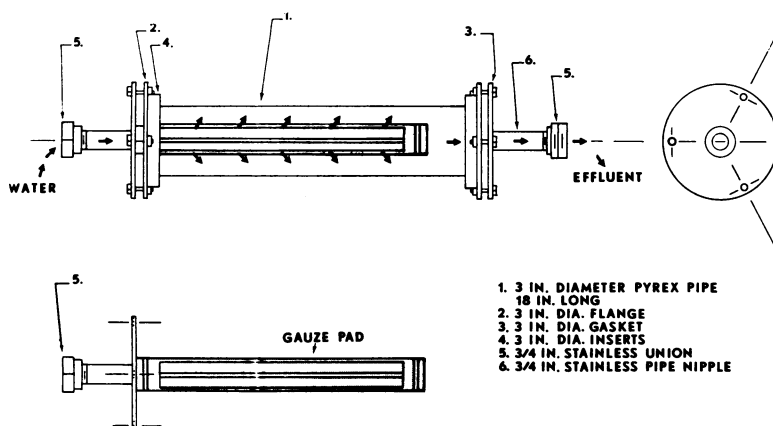


Fig. 1. Diagram of the flow-through gauze sampler.

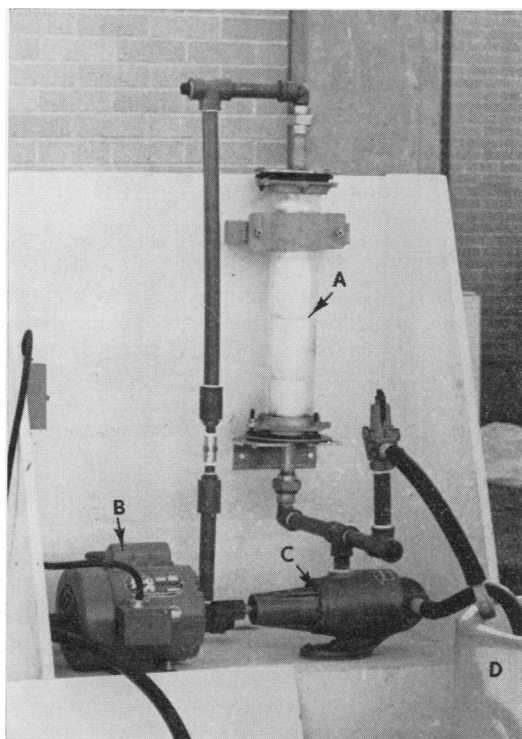


FIG. 2. Sampler in operation for experimentation. (A) Gauze sampler, (B) motor, (C) water pump, and (D) water tank.

contained approximately $10^{7.50}$ plaque-forming units (PFU)/ml.

Virus assay. Primary African green monkey kidney tissue cultures were used throughout this study. The procedures for preparation of monolayer culture and plaquing of the virus have been described (10).

Sampling device. The structure of the flow-through sampler is depicted in Fig. 1. The outer part is a Pyrex glass tube 18 inches long (ca. 45.7 cm) with a 3 inch diameter (ca. 7.6 cm). Each of the two stainless-steel end plates is equipped with a 0.75 by 3 inch (ca. 1.9 by 7.6 cm) stainless-steel nipple as the inlet and outlet for the sampled water. The gauze holder consists of two short lengths of 1.5-inch (ca. 3.8 cm) stainless-steel lip connected by four 14 by $\frac{3}{16}$ inch (ca. 35.6 by 0.37 cm) steel rods. This assembly was welded to the inlet end plate. The outlet-end pipe section was closed by welding a piece of stainless-steel sheet over the end.

Each pad was made of 16 layers of fine-mesh surgical gauze (1.45 oz/yard²; ca. 41.13/0.836 m²) weighing approximately 150 g. In between the innermost two layers, a piece of absorbent cotton weighing 100 g was inserted. This pad was used to wrap around the holder and was clamped at the lip of each end plate by a stainless-steel hose clamp. The pad over the middle section was secured in two places with string to prevent ballooning of it from pressure of water flowing through. This assembled set was autoclaved prior to installation on the wooden stand which contained a pump, with a capacity of 1 gal/min, a water meter, and plastic hoses for connecting all necessary parts. The device in operation is shown in Fig. 2.

Procedure for elution. Immediately after pumping of the sampled water, the gauze pad was removed from the device and the excessive water was allowed to drip until stopped. It was then placed in a large sterile mortar. Water in the gauze pad was pressed out with a sterile pestle and 1 N NaOH solution was added drop-wise to it until the pH of the water reached 8.0. Sufficient calf serum was added to approximate 5% of the total volume. Thereafter, the water in the mortar was allowed to be absorbed into the pad. The process of expression and absorption was repeated four to five times. Finally, the expressed fluid was collected into a graduated cylinder and more serum was added to achieve a final concentration of 5%. This constituted the first eluate.

TABLE 1. Influence of cotton on virus recovery by the gauze sampler (tap water)

Gauze type	Sample	Vol (ml)	PFU/ml	Concn factor	Total PFU ^a	Total recovery	
						PFU/pad ^a	Per cent of virus in original
Gauze alone	Original Eluate	113,560	21.4	1.0	2,430.2	16.5	0.7
	1st	150	40.0	1.9	10.0		
	2nd	100	35.0	1.5	3.5		
	3rd	100	30.0	1.4	3.0		
Gauze and cotton	Original Eluate	113,560	21.4	1.0	2,430.2	68.2	2.8
	1st	345	105.0	4.9	36.2		
	2nd	100	190.0	8.9	19.0		
	3rd	100	130.0	6.1	13.0		

^a PFU, plaque-forming units. Values to be multiplied by 10³.

TABLE 2. Effect of pH and serum on elution of poliovirus from gauze pad

pH	Buffers containing no serum		Buffers containing 10% serum	
	PFU/ml ^a	Per cent recovery	PFU/ml	Per cent recovery
Original	275	100.0	150	54.5
3.0	47	17.1		
5.0	125	44.6		
7.0	76	27.7		
8.0	40	14.6		
9.0	280	101.8		

^a PFU, plaque-forming units.

TABLE 3. Effect of successive elutions of gauze pad on viral elution (seawater)

Eluate	Run 1		Run 2		Run 3		Avg per cent
	PFU ^a	Per cent	PFU ^a	Per cent	PFU ^a	Per cent	
1st	147.4	55.6	372.6	42.9	1,968.8	59.7	52.7
2nd	56.2	21.2	172.0	19.8	605.0	18.3	19.8
3rd	34.7	13.1	160.0	18.4	430.0	13.0	14.8
4th	27.2	10.2	164.8	19.9	295.0	9.0	13.0
Total	265.5	100.1	869.4	100.0	3,298.8	100.0	

^a PFU, plaque-forming units. Values to be multiplied by 10³.

The pressed-dry pad remained in the mortar to which 100 ml of tris(hydroxymethyl)aminomethane-(Tris) (pH 8.0) containing 5% calf serum was added. The medium was absorbed into the pad which was left at room temperature for 4 to 5 min. The process of absorption and expression was repeated four to five times. The fluid was collected, constituting the second eluate. The same procedure was used to obtain the third and fourth eluates. Each eluate was stored at -20 C until assayed.

RESULTS

Effect of gauze and cotton on virus adsorption. Hoff et al. (*personal communication*) demonstrated that the combination of gauze and cotton abstracted more virus from water samples than the gauze alone. Experiments were carried out to ascertain this finding. A typical experiment follows. One sampler contained 16 layers of gauze and another, the same amount of gauze into which absorbent cotton was inserted. A total of 30 gal of tap water, containing virus of approximately 20 PFU/ml, was passed through each sampler. From each pad, three successive elutions

were made and all samples were assayed for virus contents. The results are summarized in Table 1. As shown, the pad with cotton yielded a larger volume of the first eluate. In spite of this, this pad retained approximately four times more virus than that in the pad containing gauze alone. From these results, the combination pad appeared superior to gauze alone and thus was used for all later experiments.

Effect of pH and serum on viral elution. Experiments were carried out to evaluate the effect of pH and serum content in buffer solutions on viral elutions from gauze pads. A typical experiment follows. A number of gauze pads, weighing 100 g each, were immersed in distilled water containing 275 PFU/ml overnight at 4 C. Five pads were removed from water and treated with 100 ml each of Tris buffers of varying pH levels, and the other five were treated with the same buffers containing 10% calf serum. All eluates and the water were assayed for viral contents (Table 2). The data suggest that the higher pH and addition of serum enhanced viral recovery.

Effect of repeated elutions. In most experiments

TABLE 4. *Effect of sample volume on virus recovery by the gauze sampler (tap water)*

Sample	Vol (ml)	PFU/ml	Concn factor	Total PFU ^a	Total recovery	
					PFU/pad ^a	Per cent of virus in original
Original.	37,850	15.0	1.0	567.8	38.6	6.8
Eluate						
1st.	350	5.0	0.3	1.7		
2nd.	100	112.5	7.5	11.3		
3rd.	100	162.5	10.8	16.3		
4th.	100	92.5	6.2	9.3		
Original.	113,560	15.0	1.0	1,703.4	43.1	2.5
Eluate						
1st.	340	92.5	6.2	31.5		
2nd.	100	67.5	4.5	6.8		
3rd.	100	35.0	2.3	3.5		
4th.	100	12.5	0.8	1.3		
Original.	340,680	15.0	1.0	5,110.2	95.4	1.9
Eluate						
1st.	324	150.0	10.0	48.8		
2nd.	100	280.0	18.6	18.0		
3rd.	100	132.5	8.9	13.3		
4th.	100	152.5	10.2	15.3		

^a PFU, plaque-forming units. Values to be multiplied by 10³.

presented, each gauze pad was eluted four times. The experimental data supporting such an approach are presented in Table 3. As shown, the first eluate contained approximately 50% of the number of virus particles flowing through the device. The second eluate, in general, had slightly more PFU per milliliter. Since the volume of the first eluate was larger than the second, the total virus recovery in the first eluate was always greater. The fourth eluate contained the least amount of virus, indicating that further elution is probably unnecessary for all practical purposes.

The data shown in Table 3 are from experiments using seawater as the test medium. When fresh water was sampled, the percentages of virus recovery in different eluates were comparable to those just described.

Effect of sample volume on virus recovery. The major advantage of this device is its ability to process huge volumes of water in a short time. A number of experiments were carried out to determine whether virus adsorption onto the pads would increase as the sample volume increased. The typical results from three experiments are presented in Table 4. The data indicate that an

increase in volume of the fresh water sample does not necessarily result in a concomitant increase in proportion of the virus recovered.

Experiments were also carried out to determine the effect of volume of seawater samples on viral adsorption. The results from three typical experiments are summarized in Table 5. From the values of PFU per milliliter and total recovery, virus adsorption onto the pad apparently increased as more water passed through the sampler. The number of virus adsorbed was roughly in proportion to the volume of seawater pumped through the sampler. The PFU per milliliter of the early eluates from the sampler received the largest sample, 340,680 ml (90 gal), reaching approximately 100 times that present in the original seawater sample.

Effect of NaCl on virus adsorption from fresh water. In view of the seawater data, experiments were carried out to explore the effect of addition of NaCl to fresh water samples on viral adsorption onto gauze pads. The results from two typical experiments are presented in Table 6. As shown, addition of NaCl to tap water to 3% (w/v) considerably increased the capacity of virus adsorption of the sampler. The factors of concentration and total recovery of virus from the

TABLE 5. *Effect of sample volume on virus recovery by the gauze sampler (seawater, Narragansett Bay)*

Sample	Vol (ml)	PFU/ml	Concn factor	Total PFU ^a	Total recovery	
					PFU/pad ^a	Per cent of virus in original
Original.	37,850	50	1.0	1,892.5	265.5	15.0
Eluate						
1st.	335	440	8.8	147.4		
2nd.	100	562	11.2	56.2		
3rd.	100	347	6.9	34.7		
4th.	100	272	5.4	27.2		
Original.	113,560	50	1.0	5,678.0	869.4	15.3
Eluate						
1st.	270	1,380	27.6	372.6		
2nd.	100	1,720	34.4	172.0		
3rd.	100	1,600	32.0	160.0		
4th.	100	1,648	32.9	164.8		
Original.	340,680	50	1.0	17,034.0	3,298.8	19.4
Eluate						
1st.	375	5,250	105.0	1,968.8		
2nd.	100	6,050	121.0	605.0		
3rd.	100	4,300	86.0	430.0		
4th.	100	2,950	59.0	295.0		

^a PFU, plaque-forming units. Values to be multiplied by 10³.

TABLE 6. *Effect of NaCl on virus recovery by the sampler (tap water)*

Sample ^a	Vol (ml)	PFU/ml	Concn factor	Total PFU ^b	Total recovery	
					PFU/pad ^b	Per cent of virus in original
Original (0.2) . . .	113,560	50	1.0	5,678.0	77.0	1.4
Eluate						
1st	375	100	2.0	37.5		
2nd	100	180	3.6	18.0		
3rd	100	215	4.3	21.5		
Original (3.0) . . .	113,560	50	1.0	5,678.0	2669.0	47.0
Eluate						
1st	280	5,300	106.0	1,484.0		
2nd	100	7,150	143.0	715.0		
3rd	100	4,100	82.0	410.0		
4th	100	600	12.0	60.0		

^a Values in parentheses indicate per cent NaCl.^b PFU, plaque-forming units. Values to be multiplied by 10⁵.

water samples thus treated clearly reflect the effect of NaCl on adsorption of the strain of polio-virus tested.

DISCUSSION

In this study, a continuous-flow gauze sampler designed by Hoff, Lee and Becker (*personal communication*) was evaluated for its efficiency in concentration of low levels of virus from water. It is of practical importance that virus recovery by this device was significantly increased when seawater or fresh water mixed with NaCl was sampled. The increased adsorption of even the one strain of poliovirus tested would warrant further study of this device. The merits of this device for field use are: (i) simplicity of procedure, (ii) effectiveness in sampling large volume of water, (iii) inexpensive and lack of need for sophisticated equipment, and (iv) offering of a semiquantitative assessment of the number of virus particles present in water. The volume that can be conveniently sampled by this device is considerable and surpasses that which can be processed by any known procedure. For instance, the device used in the present study can pump 1 gal of water per min. Thus 1,440 gal of water can be sampled in a 24-hr period. If the recovery rate is 20%, this device is theoretically capable of detecting several virus particles in 288 gal of water. To further increase the efficiency of this procedure, the virus in approximate 600 ml of eluates from

each pad may be more concentrated by other appropriate methods, e.g., hydroextraction, two-phase polymer separation, ultrafiltration, etc.

Such a device only offers one alternative approach potentially useful for examination of fresh or seawater, or both. It is recognized from the data presented that much work remains to be done before it can be adopted for field investigations. Further research should be done on (i) its efficiency on concentration of numerous other types of human enteric viruses, (ii) the various optimal conditions needed to attain the highest efficiency, (iii) a suitable second step for further concentrating the viruses in eluates, and (iv) engineering improvement of the device for an automatic processing of water samples in the field. From the concerted efforts, it is hopeful that a suitable method will become available in the near future.

By using the original design of a flow-through gauze sampler, Coin et al. (7) isolated numerous human enteric viruses from the raw and finished water supplies of Paris, France. Based on no information whatever on the efficiency of this sampler, Coin estimated that approximately one virus particle on the average existed in 100 gal of the Paris drinking water. In view of the data presented in this report, he definitely underestimated the degree of pollution of the Paris water. If the sampling device used in their survey had an efficiency of 1%, the Paris water should contain at least one virus particle per gal of water. The finished water supply in this country has never been examined. Whether some of our water supplies are polluted with virus remains to be determined. Chang (4) reviewed the efficacy of various steps of water treatment process in elimination of viruses. His estimation indicates that the finished water in some communities of the United States may be polluted with very low levels of human enteric viruses, e.g., several virus particles in 100 gal. Viral contaminants at this level may initiate infection in susceptible individuals. These individuals may serve as index cases from which secondary spread may be produced by personal contact. Berg (3) stated that the rare occurrence of waterborne outbreaks of enteric virus diseases in this country was probably attributable to the wide use of chlorination for water treatment.

Mosley (15) reviewed the world literature and cited 50 waterborne outbreaks of infectious hepatitis and 8 outbreaks of poliomyelitis. Recently, two hepatitis outbreaks in the northern United States were also attributed to drinking water (14, 18). Certain of these outbreaks could be traced to

faulty distribution systems or incidental contamination of water supplies with inadequately treated or raw sewage. For others, no obvious cause could be detected. Although the overt outbreaks from adequately treated water supplies are very few in this country, the possibility of water as a cause of sporadic virus diseases has gained more attention recently. To clarify the problem of viral pollution of water supplies, a sensitive and reliable method is essential. The present report is one of a series dealing specifically with this problem.

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